EXPERIMENTAL INTERSEXUALITY AND THE SEX–PROBLEM[1]

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UNTIL a comparatively recent time the sex-problem was one of the white spots on the map of biology. There is hardly another problem which has been such a playground of dilettantism, and if we look through the older literature on the sex-problem we find almost as many philosophers and economists inventing sex-theories as there are biologists. But there is scarcely another problem in biology which in the brief period of a decade and a half has emerged from a state of absolute ignorance into one of most hopeful knowledge. The all-important step which has been made during this time is the complete insight into the elementary mechanism which pro-

[1] Evening lecture delivered at the Woods Hole Marine Biological Laboratory, July 28, 1916. The majority of the facts recorded here as well as the principle of their explanation have been published in a series of papers which appeared following a preliminary report in *Sitzungsber. Ges. Morphol. Physiol. München*, 1911, namely: Goldschmidt, R., ''Erblichkeitsstudien an Schmetterlingen,'' I, *Ztschr. induct. Abstammungsl.*, 7, 1912; Goldschmidt, R., u. Poppelbaum, H., ''Erblichkeitsstudien an Schmetterlingen,'' II, *Ibid.*, Vol. 12, 1914; Poppelbaum, H., ''Beitraege zur Kenntnis, etc.,'' *Ibid.*; Goldschmidt, R., ''A Preliminary Report, etc.,'' *Proc. Nat. Ac. Sc.*, I, 1915; Goldschmidt, R., ''Genetic Factors and Enzyme Reaction,'' *Science*, Vol. 43, 1916. See further the related chapters in Goldschmidt, R., ''Einführung in die Vererbungswissenschaft.,'' 2. ed., Leipzig, 1913, and in Correns, C., and Goldschmidt, R., ''Vererbung und Bestimmung des Geschlechts,'' Berlin, 1913. A complete account of the entire work, with the necessary illustrations, is in preparation. I am greatly indebted to Dr. R. A. Spaeth for revising this MS.

duces the normal distribution of the two sexes. To-day it is well known to every one how this insight was reached practically simultaneously by the study of Mendelian inheritance and the cytological investigation of the chromosomes and how the two solutions are in most wonderful harmony. The outstanding facts which I regard as one of the corner-stones of modern biology are familiar even to the beginner in biology. Cytologically one of the sexes, the heterogametic sex, contains only one X-chromosome, the other, the homogametic sex, two of them. Every one knows how the maturation division separates entire chromosomes and therefore the heterogametic sex produces two kinds of sex-cells, *i. e.*, with and without X-chromosomes, but the homogametic only one kind, all with X-chromosomes; and how chance-fertilization produces again the two parental combinations, that is, the two sexes. You are furthermore familiar with the fact that the Mendelian experiments yield practically the same result. If we substitute the term "sex-factor" for "X-chromosome" and "hetero-homozygous" for "hetero-homogametic," the facts are identical. One sex is heterozygous for the sex-factors, say Ff; the other homozygous, say FF; the first one produces two kinds of gametes, the second only one, and chance-fertilization results in equal numbers of the parental combinations. The results of experiments on sex-limited inheritance have shown, finally, that both sets of facts are the same thing, only expressed in different language, in other words, that the X-chromosomes are the vehicles for the distribution of the sex-factors. If we state further that there are animals in which the heterozygous sex is the female, and others where it is the male, we know the elementary facts from which any further study of the sex-problem has to start.

Now that we know the elementary mechanism of sex-distribution, can we regard the sex-problem as solved? I do not think so. What are the sex-factors and how do they determine sex? Are the two sexes clean-cut alternatives and is it therefore impossible to transform one

into the other, or are they nothing but limiting points of a series, which might approach each other or even become interchanged? These and many other questions can only be approached by experimental modifications of the normal sex conditions. There is one road towards this goal, the application of the well-known influence of the internal secretion of the sex-glands upon the sex-characters in castration- and transplantation-experiments, as well as the study of analogous experiments by nature. Another approach is rather an unexpected one; this has been followed in my experiments, an account of which I now have the pleasure of giving you.

Insect-breeders have long known that in crosses of species as well as of geographic varieties a comparatively high percentage of sexual abnormalities are produced. Furthermore, any collector of moths knows that similar abnormalities, usually called gynandromorphs or hermaphrodites, appear occasionally in nature and one of the moths in which these occur is the gipsy-moth. Since this moth has a very wide range of geographic distribution through very different climates a considerable difference of geographic varieties is to be expected. Furthermore, as is well known, the sexes are extremely dimorphic. Therefore, seven years ago I began hybridization experiments of European and Japanese gipsies. I was rather fortunate in striking the right forms which gave, from the outset, the most interesting results.

The first result was that crosses of Japanese females with European males yielded normal offspring, whereas in the offspring of the reciprocal cross, European female × Japanese male, all males were again normal, but all females showed in all parts of their bodies admixtures of male characters. I first called these animals gynandromorphs. But as this term is usually applied to animals with bilateral or antero-posterior or similar mosaic of the two sex-characters, it seems advisable to use another term for these forms, which in general repre-

sent a definite step between the two sexes.[2]   The phe-
nomenon shall therefore be called intersexuality.   Further
experiments now proved that intersexuality segregates,
F_2 giving normal and intersexual animals.   It was
further shown that in some experiments the females re-
mained normal and the males became intersexual.   When
the experiments were repeated, however, different results
appeared.   And as the material used came from different
strains, suspicion arose that there are many different
races of gipsies, differing in regard to those things which
are responsible for the intersexuality.   This suspicion
was strengthened when it became probable that the pecu-
liar thing responsible for intersexuality could be influenced
by external conditions.   If this is the case, conditions in
Japan ought to be very favorable for an origin of such
racial variation, as the Japanese islands show climatic
conditions varying from an almost arctic to an almost
tropical climate.   These suppositions turned out to be
correct, as was proved by the further study of these ques-
tions in Japan in 1914 and with the material brought
from there to this country.

In order to make the results clearer and their bearing
on the sex-problem more evident, I want to apply just
the opposite method from that used in the research work,
and to give first the general interpretation of the results,
which differs only in a few points from that used since
1911, and then the experimental facts from which it has
been derived.   What we have to explain is that two
nearly related forms, both normal in regard to sex-in-
heritance, produce, if crossed in one direction, normal
offspring, in the other direction, normal males and inter-
sexual females; (2) that, as we may now say, the degree
of intersexuality is definite in a given cross, but different
in different crosses; (3) that intersexuality shows Men-
delian segregation; (4) that males may become inter-
sexual too in certain crosses.

The explanation for these and the other facts later to

2 The special meaning of this will be discussed in another paper in con-
nection with very interesting new facts.

be reported is the following: Both sexes contain the anlagen for either sex. In both sexes, irrespective of the zygotic constitution, both anlagen might become patent. Which one is to appear depends entirely upon the quantitative relation of both.[3] If we apply the usual symbols and keep in mind that the female sex is here the heterozygous one we have the following formulæ:

$$\boxed{FF}\, Mm = ♀, \qquad \boxed{FF}\, MM = ♂.$$

(It will soon be explained why the female set FF is put in a square.) The female set as well as the male set act independently and with a definite quantitative strength. In order to have a convenient term we call this quantitative value of the sex-factors their potency or valency.

[3] The idea that sex-determination is a quantitative rather than a qualitative process is of course not new. Practically all writers about the cytology of sex-determination have developed such ideas, beside the well-known old and new metabolistic theories. Such a quantitative view was proposed by myself in 1904 (''Der Chromidialapparat, etc.,'' *Zool. Jahrb.* (An.), 21) and more fully developed in 1910 (''Kleine Beobachtungen, etc.,'' *Arch. Zellf.*, 6). Other such theories were proposed by R. Hertwig, 1905–07 (''Ueber das Problem der Sexuellen Differenzierung, etc.,'' *Verhdlg. deutsche Zool. Ges.*, 1905, 1906, 1907), based on his views about the nuclear-plasmic relation. Again others of a strictly quantitative character were discussed by E. B. Wilson (''Studies on Chromosomes,'' III, *Journal Exp. Zool.*, 3, 1906), by Th. Montgomery, Jr. (''Chromosomes in the Spermatogenesis, etc.,'' *Trans. Amer. Phil. Soc.*, 21, 1906), and by Th. Boveri (''Ueber Beziehungen des Chromatins zur Geschlechtsbestimmung Sitzber. physikal. medizin. Ges. Würzburg,'' 1908–09). A full discussion of the relative values of qualitative and quantitative views in regard to sex-determination is given by Th. H. Morgan (''A Biological and Cytological Study, etc.,'' *Jour. Exp. Zool.*, 7, 1909), who decides for the latter. However, I think that all these authors no longer cling to the details of their former views. The first attempt to prove a quantitative theory of sex-determination experimentally has been made by R. Hertwig in his well-known experiments with frogs (since 1905). Quite another type of theory, practically the same as is to be used in this paper, combining the quantitative view with the Mendelian and cytological results, has been developed on an experimental basis in my papers from 1911 and 1912 (*l. c.*) and has been adopted by many writers (*i. e.*, Doncaster and Harrison, Standfuss, Witschi). A third view has since been developed by Riddle in a series of preliminary papers (Carnegie Year Book, 1913, *Science,* Vol. 39, 1914, Amer. Natur., Vol. 50, 1916, etc.). His theory is based partly on chemical studies of pigeon eggs, partly on hybridization experiments with doves. It is not impossible that in the latter something like intersexuality is produced, if I understand the short accounts thus far published.

If you like to form a definite idea you might assume that the potency means a certain concentration of enzyme which acts according to well-known laws.[4] In order to make the situation clear we assume that we are able to measure their potency. And we find that the female factorial set $\boxed{FF}$ is 80 units strong and every male factor $M = 60$ units. Then in the female formula $\boxed{FF}$ Mm the female set overpowers the one M present by 20 units, whereas in the male formula $\boxed{FF}$ MM the two Ms with the value of 120 are 40 units stronger than $\boxed{FF} = 80$. Now we face two possibilities. Either the slightest preponderance of one set over the other, say by only one unit, is sufficient to determine the male or female sex, or there is a definite minimum of preponderance necessary—we call it the epistatic minimum—beyond which one or the other sex appears. Let us now suppose this minimum to be 20 units. Then of course 40 units are left between the two extremes male-female preponderance. If we call the difference value between the male and female factorial set e, then we have a female, when $\boxed{FF} - M = > 20$ and a male, when $MM - \boxed{FF} > 20$, or in other words the limiting values for e for the two sexes are $+20$ and -20. We can now express this conception graphically in the following diagram, where the values

Fig. 1

of e are arranged on a straight line. Individuals to the right of $+20$ are females, to the left of -20 are males. But what of the intermediate points? These are the intersexual animals; if they are heterozygous for M they are intersexual females and if they are homozygous for M they are intersexual males. How does this diagram now

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[4] For proof that this conception comes near the truth see Goldschmidt, R., "Genetic Factors and Enzyme Reaction," *Science*, N. S., 43, No. 1099, 1916. Very important new facts will be published later, which will probably enable us to replace the symbolistic Mendelian language, used here, by more definite physico-chemical conceptions.

explain the fundamental experiment? Suppose we have
two races both normal in regard to the quantitative be-
havior of their sex-factors, but with different absolute
values of the potencies. The values are supposed to be
the following:

Weak European Race	Strong Japanese Race
♀ $\boxed{FF}$ Mm	$\boxed{FF}$ Mm
80 60	100 80
♂ $\boxed{FF}$ MM	$\boxed{FF}$ MM
80 60 60	100 80 80

It is evident that both races, if bred true, behave nor-
mally. Here we must now add that the female factors
or anlagen are inherited exclusively maternally, without
any paternal influence. Therefore the square. But the
M's are typical Mendelian sex-factors. Let us cross now
a Japanese ♀ with an European ♂. F_1 is then

$$F_1 \,♀ \quad \boxed{FF}\ \text{Mm,} \qquad F_1 \,♂ \quad \boxed{FF}\ \text{MM.}$$
$$\quad\quad 100 \qquad 60 \qquad\qquad\quad 100 \quad 80 \quad 60$$

The value e is then $+$ and $-$ 40, the offspring is normal.
The reciprocal cross European female with Japanese
male gives

$$F_1 \,♀ \quad \boxed{FF}\ \text{Mm,} \qquad F_1 \,♂ \quad \boxed{FF}\ \text{MM.}$$
$$\quad\quad 80 \qquad 80 \qquad\qquad\quad 80 \quad 80 \quad 60$$

Now we see that in the female e or $FF - M = 0$. The
animal is intersexual, exactly half-way between male and
female.

Instead of deriving further theoretical expectations
we shall now see how the experimental facts fit these
general conceptions of a quantitative nature of sex-de-
termination. The first point is of course the question of
the different absolute and relative potencies. There are
primarily two ways open for testing it. One is to influ-
ence these potencies experimentally. Only a few pre-
liminary steps in this direction have thus far been made,

which may be omitted here. Another way would be to find many races which differ constantly in regard to the potency of these factors, which could be shown by the results of cross-breeding. The expectations are, then, that with increasing value of M, in crosses, where these races are used as males in combination with "weak" females, a corresponding type of female intersexuality must appear, giving a complete series from femaleness to maleness. And if we can find a race with so high a potency of M, that the combination lies beyond the epistatic minimum for maleness, then all the would-be females will be transformed into males. I now have at hand such races from Europe and Japan and can produce at will and in 100 per cent. of the offspring all grades between the two sexes. Thus we have one Japanese race, the race G, medium strong in regard to the potency of the factor M. If we cross these males with females of the Japanese race K, which shows comparatively low potency of the female factorial set, all would-be females in F_1 are slightly intersexual. We might put them at the point + 15 in our diagram (Fig. 1). Their antennæ are feathered, but less than in males, a portion of the wings assumes the brown color of the male, there are not as many eggs as in a normal female, but the mating instincts as well as the copulatory organs are still female and the eggs may be normally fertilized. Then there is a European race F and a Japanese race H, both with a still lower potency of the female factorial set. If we cross these with the same Japanese males G we get somewhat slighter female intersexuality. All secondary sex-characters are more male-like; the instincts are still female and the animals attract the males and mate. Then one of the characteristic hairy egg sponges is laid, but it contains no eggs, only hairs. The copulatory organs are already changed in the direction of the male and no successful mating and egg deposition is possible, although the abdomen is filled with ripe eggs. Then there is another European race F with very low potency of the female factors. If we mate these with the same males G,

intersexual females appear which are more than half-way between males and females. The secondary sex-characters are almost male. The instincts and behavior are about intermediate between the sexes. Males are scarcely attracted or not at all and no mating occurs. The copulatory organs show the strangest combinations of the male and female types, but there are still typical but rudimentary ovaries left. There is now another Japanese race in my possession, the race X. This one exhibits a still higher potency of the male factor M. If we cross this one with the European race F, a still higher degree of intersexuality appears. Now we have animals which externally are almost indistinguishable from true males. But certain characters, especially in the copulatory organs, still show their female origin. The instincts are entirely male and they try—always unsuccessfully—to mate with females. But the most interesting feature is the sex gland. This is a body looking externally like a testis, but showing in sections every single step between an ovary with nothing but immature eggs through a mixture of ovarial and testis tissue to a real testis. This is of course the highest grade of intersexuality which can be reached. The next step would be the complete transformation of the would-be females into males. And this can be obtained too. I have two Japanese races O and A which show such a high potency of M that crossed with any European females and the Japanese females H they produce nothing but males, all would-be females being converted into males.[5]

[5] It might be added here that two different lines of female intersexuality can be distinguished in regard to the most conspicuous feature, the color of the wings. In one line intersexuality begins with white wings, then dark cunei appear on the wings; they grow larger and larger, forming streaks along the veins until finally only fine white spots are left on a dark wing. The second series shows even in the first grades of intersexuality male color all over the wings without any streak formation. Which type appears depends on both races involved, and is due to physiological conditions in regard to pigment formation which are not yet entirely clear. The intersexual males always exhibit the first type. Color photographs of an almost complete series of the second type are given in my papers from 1912 and 1914. Photographs of the first type in the female series are not yet published. A complete series of the transformation of the female copulatory

I think these facts alone would be sufficient to prove
that the above given quantitative conception of sex-de-
termination is the right one.   But we can quote further-
more a real experimentum crucis.   We have seen that the
very weak European race F crossed with the medium
strong Japanese males G give a fairly high grade of
intersexual females.   In our diagram of the values of e
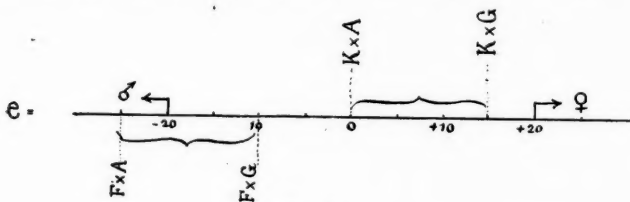we might put them at the point — 10 (Fig. 2).   The same

Fig. 2

weak females crossed with the very strongest males A
gave nothing but males, the would-be females standing
now on the point, say —25.   Now the strongest of the
weak races in regard to FF, *i. e.*, the Japanese race K,
gave with these same males G slight female intersexual-
ism, say at point +15.   If our conception is right, the
same females, crossed with the very strong males A,
ought to give medium intersexual females, to be put at
the point 0.   And this is the actual result.[6]

Now we have reached a point where we want to know
how the complexes FF and M are inherited.   Anybody
familiar with Mendelian analysis knows which results are
to be expected if F and M are independent Mendelian
factors.   These tests, so far as fertility allows them to be

organs based on our material is pictured in the paper of Poppelbaum
(*l. c.*). Photomicrographs of the transition from an ovary to a testis are
given in my paper of 1914.   In regard to the occasional appearance of a
single normal female in cultures where all females are transformed into
males, see our publication in the *Proc. Nat. Ac.*   It was supposed that they
originated from a case of ''non-disjunction.''   Their behavior in breeding
does not agree with this supposition but suggests another explanation, which
at the same time explains the occasional appearance of intersexual males
in nature.   Details can not be given without a lengthy discussion.

[6] It might be added that a similar experiment can be performed with
the Massachusetts race, which behaves about like the Japanese race K.

made, prove, of course according to expectation, that the factor M is Mendelian and carried in the X chromosome. All results of F_2 and back crosses agree in this point. But what is $\boxed{FF}$? Originally I believed that I could prove its Mendelian character too. This was a mistake, produced by the interference of another phenomenon, connected with the wing-color inheritance. The experiments made since my first communications prove thus far that the complex $\boxed{FF}$ is inherited only through the mother, maternal grandmother, etc., *i. e.,* in the protoplasm of the egg.

We are now prepared to consider the production of the intersexual males, thus far omitted in our discussion. A series of them has been produced extending almost to femaleness. They are easily distinguishable from intersexual females (a point which is of special interest; one explanation being that factors contained in the Y chromosome are responsible for it). The intersexual males always exhibit the first of the above-mentioned (foot-note page 713) types in regard to wing coloration, white streaks appearing on the dark wings. While the wings assume more and more the female shape, the dark color becomes confined gradually to the wing venation, and in the extremest types thus far bred only a few brown spots appear upon some veins. All the other characters, like size of abdomen and copulatory organs, change hand in hand with this. But the behavior of the sex glands is not yet clear. In low grades of male intersexuality the testis always contains some ovarial tissue.[7] But the highest grade intersexual males of almost female exterior contained a paired[8] sex gland of somewhat testis character, filled with giant bundles of apyrene spermatozoa, and containing no eggs.

If we now deduce from our general interpretation how these interesting males might be produced, we realize that

[7] See picture in Poppelbaum's paper. The lower grades of male intersexuality are photographed in our papers from 1913 and 1914. Pictures of the higher grades are not yet published.

[8] The ripe testis is an unpaired organ.

they are expected to appear when $\boxed{FF}$ is comparatively high in potency and MM comparatively low. There are two possibilities for this event. (1) If we revert to our original example of a cross between weak European and strong Japanese races, we have in one direction:

$$\text{Eu. } \female \times \quad \text{Jap. } \male$$
$$\boxed{FF} \text{ Mm} \times \boxed{FF} \quad \text{MM.}$$
$$\quad 80 \quad 60 \qquad 100 \quad 80 \ 80$$

As $\boxed{FF}$ is inherited maternally, any generation of this cross will have the weak set $\boxed{FF} = 80$. There is no combination of two M's possible which is not at least 20 units higher than FF, therefore no intersexual males can occur. Now take the reciprocal cross:

$$\text{Jap. } \female \times \quad \text{Eu. } \male$$
$$\boxed{FF} \text{Mm} \times \boxed{FF} \quad \text{MM.}$$
$$\quad 100 \ 80 \qquad 80 \quad 60 \ 60$$

The F_1 males are $\boxed{FF} \quad$ MM and therefore normal, as
$$\qquad\qquad\qquad\qquad\quad 100 \quad 80 \ 60$$

$e = -40$. F_2 from this cross has again the maternal and grandmaternal set $\boxed{FF} = 100$. The Mendelian factors MM are recombined and we get the combinations:

$$\text{MM} \quad \text{and} \quad \text{MM.}$$
$$80 \ 60 \qquad\qquad 60 \ 60$$

The latter males are therefore:

$$\boxed{FF} \quad \text{MM.}$$
$$100 \quad 60 \ 60$$

This means that e has just the limiting value -20. It follows, that if we have two races, in which the relative values differ only slightly from this example to the disadvantage of the weaker M, say $M = 59$ instead of 60, male intersexuality is to be expected in the F_2 generation of a cross, where the mother belongs to the stronger race. This is indeed one of the actual facts.

According to the above derived formulæ these intersexual males ought to number exactly one half of the

male individuals. But this has so far never been the
case. In order to understand it we have to point now to
a fact which we omitted in our previous discussions. We
have located the intersexual animals, males and females,
at a certain point between the two sexes, as represented
in the two diagrams. But in fact these points are only
the mean of a certain range of variation in regard to the
grade of their intersexuality. Whether this means that
the potencies of the factors are variable or only their
ultimate effect shows variation produced through influ-
ences during the development, is a question not to be
discussed here. The fact is, however it might be caused,
that the value of e as the measure of intersexuality, is
variable around a mean. If this is the case, the following
expectations are to occur (Fig. 3): If the values of e
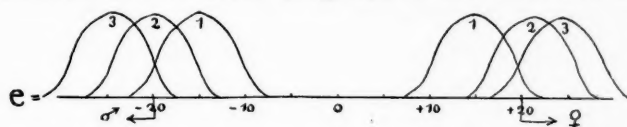
FIG. 3

approach the epistatic minimum (20 and — 20 in our dia-
gram), then a point must be reached where the vari-
ability-curve stretches beyond this limit. That means—
curve 1—that in cases of low grade intersexuality some
+ or —·individuals might overlap into the normal. Of
course any position of these curves and a corresponding
numerical relation of normals and very slightly inter-
sexual animals might be met with, as the curves 2 and 3,
Fig. 3, indicate. And indeed such cases have been found
as well on the female as on the male side. Types of this
kind, realized in the F_2 crosses with male intersexuality,
are those with which we have dealt in the previous para-
graph. Thus far all facts agree with this conception, in
one culture up to F_5. Further checks are in progress.

But there is another possibility for the production of
intersexual males. Let us assume that we could find two
races of the following constitution:

Strong race ♀ = $\boxed{\text{FF}}$ Mm   ♂ = $\boxed{\text{FF}}$ MM,
                       100 60   100 60 60

Weak race $♀=$ ⟨FF⟩ Mm $\qquad ♂=$ ⟨FF⟩ MM.

80 50 $\qquad\qquad$ 80 50 50

The F_1 cross of these, using the strong race as mother, would give:

F_1 ♀ ⟨FF⟩ $\qquad$ Mm $\quad =$ normal female,

100 $\qquad$ 50

F_1 ♂ ⟨FF⟩ $\qquad$ MM $\quad =$ intersexual male, as $e = -10$.

100 $\qquad$ 60 50

Every single male ought to be intersexual. Through the kindness of Dr. Machida in Tokyo, who first has reached this strange result, I could bring back from Japan two such races and they breed exactly according to expectation.

This is about the skeleton of our work around which of course many interesting experimental, morphological, embryological, cytological and physiological facts are grouped. I am rather optimistic in regard to the general conclusions, which might be drawn from these facts as well as regards the sex-problem as on some fundamental questions of heredity. Combining these facts with the work on hormone action as related to sex we can, I think, form a pretty clear idea about sex-differentiation and determination.[9]

If we put them in line with the facts of experimental embryology concerning the determination problem we see the outlines of a promising theory of heredity. But this can be discussed only when a full account of the work has been given.

[9] The relation of gametic or zygotic intersexuality to the hormonic intersexuality produced in castration and transplantation-experiments in Crustacea, birds and mammals (especially the work of G. Smith and Steinach) to which now, after F. Lillie's discoveries, the case of the free-martin has to be added, has been worked out in the chapter on Sex in my treatise on genetics (*l. c.*), 2d edition. A detailed comparison between the moth-work and the facts known about human intersexuality, containing, too, a discussion of the relations of zygotic and hormonic intersexuality, was sent to Germany for publication about half a year ago, but it is not known whether it has been published there or adorns the waste-paper basket of His Britannic Majesty's censor.

PIEBALD RATS AND MULTIPLE FACTORS

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Introduction

The experiments of Castle and Phillips (:14) with pie-
bald rats afford the largest mass of recorded data on the
influence of selection in mammals. For 17 generations,
the area of pigmentation on their hooded rats has been in-
creasingly modified. In one line (the plus race) the pig-
mentation has been extended; in the other line (the minus
race) it has been reduced. When rats from the plus or
minus race are crossed with fully pigmented rats, such as
the normal wild, or the Irish variety, the hooded pattern
behaves as a simple Mendelian recessive, disappearing in
the first generation and reappearing in one fourth of the
offspring in the second generation. These results lead to
the conclusion that hoodedness appears when a certain
germinal unit, or factor, is in a zygote in a homozygous
condition. Besides this, Castle concludes that the factor
determining hoodedness fluctuates, and, in accord with its
fluctuations, the amount of hooding varies. It follows
that the selection of extreme grades of hoodedness results
in the simultaneous selection of extreme variations of the
factor. Moreover, Castle (:16, p. 722) finally concludes
that the selection of these extreme grades of hoodedness
influences the direction in which the factor for hoodedness
varies.

These conclusions bear on one of the most generally in-
teresting and vital questions before biologists. If, be-
sides deciding which individuals shall mature and repro-
duce, selection can influence the direction in which the
units of inheritance, or factors, vary, there can be no
question but that

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selection, as an agency in evolution, must then be restored to the important place which it held in Darwin's estimation, an agency capable of producing continuous and progressive racial changes (Castle, 15*b*, p. 97).

Castle's experiments have justly become famous. For eight years they have been continuously in progress; they have involved large amounts of arduous labor; they have been conducted with unflagging zeal and high ideals of scientific attainment. The conclusions drawn from such an important investigation should receive painstaking consideration.

The writer has been conducting selection experiments which have led him to conclusions different from those reached by Castle. Although these experiments have not involved the expenditure of so much labor and time as did Castle's work, they include three times as many generations and four times as many individuals as are reported by Castle. One investigation was on rats, the other on flies, yet there are so many similarities in the results that the writer was led to make a careful analysis of Castle's papers in an attempt to discover the basis for the conflicting conclusions. The final result of this study was to make the writer feel that the following statements in regard to the hooded rats are too positive.

All the evidence we have thus far obtained indicates that outside modifiers will not account for the changes observed in the hooded pattern, itself a clear Mendelian unit (Castle, :15*b*, p. 722).

. . . there can be no doubt that only a single genetic factor is here involved (Castle, :16, p. 95).

It is precisely this last named category of cases [a single factorial basis undergoing quantitative variation] which alone can explain our rat results (Castle, :15*b*, p. 725).

Energetic attacks have been made on the interpretation Castle has given to his results, and certain unwarranted criticisms have been duly answered. That the theory of multiple factors may be applied to the results as published in 1914 in the Carnegie Institution Publication No. 195 was indicated therein by Castle, and further emphasized by Muller (:14). Most of the criticisms of the experiments with the hooded rats have been based on the

generalizations that have been made, and not directly on the data. In this paper the writer has used the original data, making verifications where possible, and recalculations of many of the constants. A few inconsistencies and arithmetical errors were found.

That there may be no misunderstanding as to the nature of the multiple factor interpretation, the following scheme is suggested. In the absence of any factor that determines uniform color—in other words, in the presence of two doses of the factor for hoodedness—the amount of pigment on hooded rats may be influenced by several factors. Some of these increase, others reduce, the pigmented area. The factors that increase the pigmented areas (plus factors) form Mendelian pairs (allelomorphs) with the factors that decrease the pigmented areas (minus factors). Dominance is lacking; if a factor is contributed to the zygote by both parents, that factor has more power than if it had come from only one parent. Furthermore, environment, or other conditions which are not inherited, being outside the germ plasm, have such a modifying influence on the pigmented areas that the potential differences between individuals determined by different combinations of factors in the germ plasm, are frequently concealed. It is not pretended that this is the only application of the multiple factor hypothesis that can be made, but it is hoped that the following arguments may become more significant with this suggested application in mind.

The writer herein undertakes to show that the conception of multiple factors may still be applied to Castle's data. The points that favor the multiple factor interpretation of the rat experiments, as well as certain objections that are said to definitely disprove this theory, are brought together in the following paragraphs.

POINTS FAVORING THE MULTIPLE FACTOR INTERPRETATION

1. The gradual divergence of the plus and the minus races may be brought about by the sorting out of groups

of different factors. It has been generally recognized that this is a possible conception. The following authors have considered this point: Castle and Phillips, :14, Muller, :14, Hagedoorn, :14, MacDowell, :15.

2. The hybrid ancestry of the original parents affords a source for a large amount of heterozygosis. It is the reduction of this heterozygosis that selection is supposed to accomplish, in separating the two races, plus and minus.

3. Such a reduction of heterozygosity would be hindered by the large number of matings made between rats less closely related than brother and sister. This point has been discussed by Muller.

4. By breaking the correlation between the soma and the germ plasm, environment has probably played a large part in hindering the reduction of heterozygosity. Apparently it has been assumed that there is a close relationship between the germ plasm and the soma, that the smooth curve of the averages in successive generations proves that the germinal variations, to which the rise in the curve is due, are small and constantly occurring. But, since the rôle of environment is not known, the gradual advance in the averages can not prove anything as to the size of the germinal variations. The presence of regression makes it clear that environmental, or extra-germinal, influences are active in producing variability in the hooded pattern. Regression is really a measure of the degree of independence of the soma and germ plasm. Regression expresses the inverse relationship between the actually tested breeding possibilities and the appearance of the parents. There can be no question as to the activity of environmental influences; of their power and nature nothing seems to be known. The immediate environment of the undifferentiated blastomeres is probably as important a factor in the final appearance of a character as the germ plasm itself. The factors in the germ plasm are like chemicals that will react in a definite way in connection with certain other chemicals; when

different ones are combined with the first ones, the results may be reversed. Now to study one variable (germ plasm) through a measure (soma) influenced by a second variable (environment) will seldom give correct results if the effect of the second variable is not clearly recognized and discount made for its influence. In the present case, it appears that the curve of the averages can only show the degree to which the variations due to environment and the germinal variations tend to go in the same direction. That there is a rise in the curve shows that, on the average, they are a little more likely to agree in direction than to contradict each other. On the other hand, since the environmental variations can not be accounted for and eliminated, the curve gives no information as to the actual or relative potencies of either set of variables. That there are no fluctuations in the curves may have been assumed to prove that environment is constant and therefore does not demand consideration. But this conclusion can not be safely drawn from the facts. The curves probably do mean that, when generation is compared with generation, the variations of the environment are cancelled out; they mean that these environmental, or extra-germinal, variations occur within a generation, and probably within a family or within the gonads of the parents. Environment might well be ignored were the ultimate question to be answered, ''How much can selection change the average grade of hooded rats?'' But this is not the main question. The question to be answered is, ''What is the nature of the changes in the germ plasm?''

In view of all this, one can find slight justification for assuming that the germinal variations were small and constantly occurring. It seems entirely possible that the environmental, or extra-germinal influences were strong, perhaps even more effective than the germinal constitution. In this case, there would be no need to assume a very large number of factors to find a multiple factor explanation for the slow advance wrought by selection.

Such strong environmental influences would, for the most part, effectively confuse the various combinations of germinal factors, and selection would continue to produce slight advances for a long time.

5. Castle has explained (Castle and Phillips, :14, p. 24) the significance of the ". . . observed reduction of variability" for the multiple factor interpretation; he stated at the same time that ". . . extensive modification through selection is possible without any marked falling off in variability." Since the observed reduction
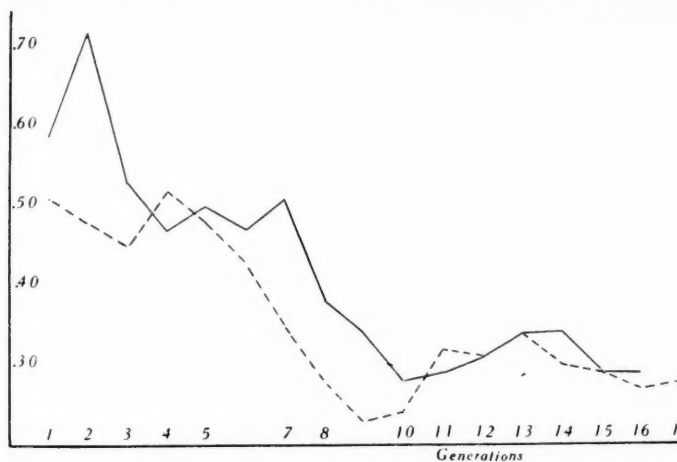
Fig. 1. Standard deviations of the plus race (solid line) and minus race (broken line) in the various generations of selecting. Ordinates represent standard deviations in terms of grades of hooding; abscissæ represent generations of selecting. Calculations made from the data as given by Castle and Phillips, 14, in Tables 1–13 and 16–28.

in variability is not considered to be marked, Fig. 1 is presented to show the facts graphically. The standard deviations plotted in this graph have all been calculated directly from the data, and in several cases they differ slightly from those given by Castle. The decrease in variability that is shown by this figure is the expected result of reduced heterozygosity accompanying continued selection.

6. The question of the rate of advance has been an-

swered by the statement that "no slowing up is observable in the rate of change of the racial character under selection either plus or minus " (Castle, :16, p. 96).   This is assuredly a very vital point in the contention that multiple factors will not explain the results.   For, if the rate of advance has not fallen off, and if, during seventeen generations, each selection has been as effective as the preceding one, it certainly would look as though this progress were due to constantly varying germ plasm, and not to the sorting out of certain groups of factors.   Were a sorting out of factors going on, each advance would restrict the possibilities for further advances, so that in a series of selections the rate of advance would decline.

In Castle's "Heredity," page 122, Fig. 41 are shown the curves of the averages of the first eight generations of the plus and minus races.   These curves begin with the average of the offspring that appeared after the first selection.   From this point on, the advance shown by the curves is gradual.   But should not the advance resulting from the first selection be recorded?   The average of the first selected generation was not the point of departure. To show the advance resulting from the first selection, the first point of the curve must give the average of the hooded race before the first selection.   Unquestionably the difference between the average of the unselected race and the first selected generation was an advance due to selection, yet this advance is apparently ignored in the statement quoted above, as well as in the figure cited. The first selection resulted in a very much greater advance than any other single selection in the whole series. It took the ten subsequent selections to separate the means of the two races as far as the first selection separated them.   If each selection had produced a like advance, the eleventh generation of selection should find the averages of the two races eleven times as far apart as they were after the first selection instead of twice as far apart. Failure to consider the advance due to the first selection has concealed one of the most striking features of the

whole series of experiments, namely, that the first selec-
tion brought about an immediate and abrupt establish-
ment of two races with means 3.05 grades apart.   The
greatest divergence between the two races due to a single
selection in all the following generations was 0.64 grade.
This followed the third selection.   In the second genera-
tion there was a reduction of the average of the plus race.
Castle explains this as follows:

To obtain larger numbers of offspring, several new pairs were added
to the experiment in this generation which did not appear in Table I
either as offspring or parents, but which were derived from the same
general stock as the parents of generation one (Castle and Phillips, :14,
p. 9).
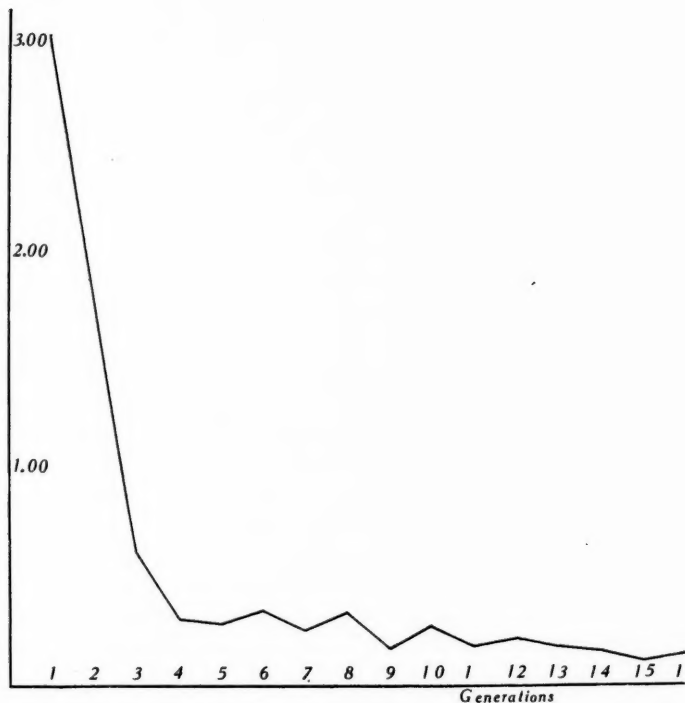
FIG. 2.   Rate of divergence of the means of the two selected races in the
various selected generations.   The point for the second generation has been
omitted from the curve; see explanation in text.   Ordinates represent grades of
hooding; abscissæ represent generations of selecting.

After the third generation there is, in general, a gradual decline in the effectiveness of selection, till in the fifteenth generation the advance is 0.12.   In support of this statement, which stands in direct disagreement with the quotation at the head of this section, Fig. 2 is offered.   In this figure the ordinates represent the increases in the differences between the two racial means in successive generations.   Since the decline in the second generation, as explained, seems to have no immediate significance, this point has been omitted from the curve.   The advance in the third generation has been calculated from the difference between the two races in the first generation, which of course gives a slightly smaller advance than if the difference in the second generation had been used. The greater part of the falling off occurs in the plus race, but both races show the same general tendencies, namely, a sudden advance as the result of the first selection, with much reduced advances following subsequent selections. It may be supposed that there was a greater degree of heterozygosity in the parents selected to start the minus race than in those selected to start the plus race.   This might explain the smaller initial advance in the minus race (one unit as compared with two in the plus race) as well as the more prolonged and slower subsequent advances.

7. Regression, as Castle uses the term, is the difference between the averages of the selected parents and their offspring.   It is due to the imperfect correlation of two variables, intra-germinal and extra-germinal differences, and so, as stated, it forms a gauge of this relationship. Its amount will be reduced by a reduction in the amount of variability of either variable.   In the later generations the regression is reduced.   We see no reason to suppose that environment as a whole acts any differently in different generations.   Therefore this reduction in the amount of regression becomes further evidence in support of the supposition that the germ plasm is more uniform, more homozygous in the later generations.

8. As Muller has correctly reasoned, successful return selections would be expected on the multiple factor view, supposing the races were still "heterozygous even after generations of selection" (Muller, :14, p. 571). It may be added (MacDowell, :15, p. 95) that the failure of a return selection to reduce the average, as long as the advance selection was progressing, would be strong evidence against the multiple factor interpretation. As long as there remained any heterozygosity in the race, both advance and return selection should succeed in moving the averages.

9. In no case should return selection lower the averages at a rate faster than advance selection was raising them at the same time. That is, a certain degree of heterozygosity will permit a certain rate of advance or decline of the averages. The return selections that were started after several advance selections did not show a decline that could be compared with the sudden advance that occurred in the first selected generation. The plus selections had reduced the heterozygosity and had thus set closer limits on the effectiveness of return selection, as well as of further advance selection. So return selections from later generations should be less effective than return selections from the earlier generations. In the plus race, generation 7, there is a difference of .84 between the average of the offspring of rats selected to continue the plus race and the average of the offspring of rats chosen for a return selection. In generation 12 the corresponding difference is .60. In the minus race the difference between the averages of offspring from high and low grade parents in 7, 8 and 9 average .50. In generation 12 the difference is .28. The numbers of rats are very small in most cases, but it is interesting to note that as far as they go they seem to show that return selection is less effective in the later generations than in the earlier ones.

10. Castle has shown that the increase in variability in the first generation of a cross between the plus and minus races may be considered an indication of segrega-

tion, therefore, of heterozygosity in the races (Castle and Phillips, :14, p. 30).   Muller (:14, p. 573) repeats Castle's suggestion that the further increase in the variability in the second generation of this cross argues for the supposition that the two races differ in regard to several factors.   This is a logical interpretation uncontested by the facts, though of course it is not proved.   On the other hand, it has been proved, by the crosses between the two races, that they are not distinguished by just one Mendelian unit.   Now if the increased variability in the F_1 of the cross between the plus and the minus races be due to heterozygosis in these races, and if selection is reducing this heterozygosis, crosses made after the races had been selected for a longer time should give less variability in F_1.   The figures show this to be the case.   If there is less variability in F_1, in later generations there should correspondingly be less variability in the F_2.   We find:

F_1 from cross plus by minus, generations 5 and 6 = S. D. 0.71; generation
    10 = 0.60

F_2 from cross plus by minus, generations 5 and 6 = S. D. 1.01; generation
    10 = 0.87

11. The reductions in the averages of both the plus and minus races after crossing with wild or Irish, first led Castle to consider a factorial interpretation (Castle and Phillips, :14, p. 25).   Muller (:14, p. 574) has fully restated the bearing of this on the multiple factor theory. The cross has apparently undone selection to some extent by restoring some of the factors that had been selected out in forming the two races; the cross has increased the heterozygosity of the extracted hooded rats, returning plus factors to the minus race and minus factors to the plus race.

In the light of the above interpretation, the conversion of the minus race into the plus race by means of a cross is significant.   Selection for increase in pigmentation was started from extracted hooded rats from a cross of minus with wild.   The first generation of this selection made as sudden an advance as the first generation of selected plus rats did at the beginning of the experiment.

It is to be observed that a cross makes a profound difference in the effectiveness of return selection. Crossing has so modified the germ plasm that rats from the minus race immediately, without any gradual return to the "0" grade, repeated the history of the plus race. Further, plus selection was carried on in this new race. Castle (Castle and Phillips, :14, p. 21) emphasizes the fact that this race is free from the objection urged against the main experiment, namely that the closest inbreeding was not carried out. Further interest in this closely inbred race lies in the fact that, although it starts out with a curve almost identical with the first generations of the plus race, the rate of advance falls off faster than it does in the main plus race. One may suppose that the cross produced an F_2 in which some rats had a degree of heterozygosity similar to that which existed in the original unselected stock; a closer inbreeding reduced the heterozygosity more rapidly.

12. The earlier generations of the plus race when crossed with wild are only slightly reduced in pigmentation. In Table 43, Castle and Phillips (:14, p. 48) show, among other things, the averages of hooded grandchildren extracted from a cross with Irish. In comparison with these are placed averages specified to be of offspring from the same grade parents and the same generation of the uncrossed selected race. References to the proper tables of the uncrossed selected races show practical agreement with the averages as quoted in this Table. In Table 42, which gives corresponding results of crosses with wild, three of the averages of the uncrossed races are taken from the same generation as the parents crossed, and three seem to be taken from the following generation. It is a matter of importance to have correct standards for judging the modifications due to crossing. There might be a question whether one should use the average of the generation from which the parents came, or the following one; but in either case the use should be constant. Although the averages of

the generations from which the parents came have been used for comparison in eight of eleven crosses, it appears to be a more fair procedure to compare the averages of the offspring produced by parents of the same grades and generations as used in the crosses. Suppose the hooded parent crossed was grade 2, from the fourth generation, then the average of the *offspring* from parents of grade 2 from generation 4 should be compared with the hooded offspring in F_2. In other words, the average to be used for comparison would be found in generation 5. On this basis the comparisons shown in Table I have been made. Returning now to the statement at the head of this section, that when crossed with wild, the earlier generations of the plus race are only slightly reduced in pigmentation, this table shows that, when third generation parents were crossed, the extracted hoodeds were lowered .04; when fifth and sixth generation parents were crossed, the lowering of their hooded grandchildren was greater, .17; when the hooded parent came from the tenth generation, the average of the hooded grandchildren was lowered .76.

TABLE I

CALCULATION MADE FROM DATA FROM CASTLE AND PHILLIPS, :14, TO SHOW THE EFFECTS OF CROSSING ON THE AVERAGES AND STANDARD DEVIATIONS OF THE EXTRACTED HOODEDS

Numbers in brackets are those given by Castle and Phillips.

A. *Comparisons of the Averages of Extracted Hooded Rats with the Averages of the Offspring of Hooded Rats of the Same Grade and Generation as the Hooded Rats Used as Parents in the Various Crosses.*

	Wild by Minus Race		
	2½	6	10
Generation from which hooded parent came			
Average grade of F_2 hoodeds	+ .31	+ .25	+ .25
Average grade of uncrossed hoodeds	− 1.18	− 1.72	− 2.12
Average grade of uncrossed hoodeds as published	(− 1.20)	(− 1.59)	(− 2.05)
Raised by cross	1.49	1.97	2.37

	Wild by Plus Race		
	3	5 + 6	10
Generation from which hooded parent came			
Average grade of F_2 hoodeds	+ 2.56	+ 2.97	+ 3.15
Average grade of uncrossed hoodeds	+ 2.60	+ 3.14	+ 3.91
Average grade of uncrossed hoodeds as published	(+ 2.60)	(+ 3.14)	(+ 3.84)
Lowered by cross	.04	.17	.76

	Irish by Minus Race		
Generation from which hooded parent came	3½	4	7½
Average grade of F_2 hoodeds	— .62	— .73	— .94
Average grade of uncrossed hoodeds	— 1.28	— 1.64	— 1.83
Average grade of uncrossed hoodeds as published	(— 1.31)	(— 1.18)	(— 1.75)
Raised by cross	.66	.91	.89

	Irish by Plus Race	
Generation from which hooded parent came	2	3
Average grade of F_2 hoodeds	+ 1.27	+ .95
Average grade of uncrossed hoodeds	+ 2.10	+ 2.60
Average grade of uncrossed hoodeds as published	(+ 1.80)	(+ 2.50)
Lowered by cross	.83	1.65

B. *Comparisons of the Standard Deviations of Extracted Hooded Rats with the Standard Deviations of the Offspring of Hooded Rats of the Same Grade and Generation as the Hooded Rats used as Parents in the Various Crosses.*

	Wild by Minus Race		
Generation from which hooded parent came	2½	6	10
S. D. of F_2 hoodeds	1.03	.90	1.18
S. D. of uncrossed hoodeds	.56	.33	.31
S. D. of uncrossed hoodeds as published ...	(.49)	(.44)	(.24)
Increased by cross	.47	.57	.87

	Wild by Plus Race		
Generation from which hooded parent came	3	5 + 6	10
S. D. of F_2 hoodeds	.50	.52	.45
S. D. of uncrossed hoodeds	.47	.47	.29
S. D. of uncrossed hoodeds as published	(.53)	(.49)	(.36)
Increased by cross	.03	.05	.16

	Irish by Minus Race		
Generation from which hooded parent came	3½	4	7½
S. D. of F_2 hoodeds	.64	.60	.84
S. D. of uncrossed hoodeds	.53	.34	.26
S. D. of uncrossed hoodeds as published	(.48)	(.46)	(.35)
Increased by cross	.11	.26	.58

	Irish by Plus Race	
Generation from which hooded parent came	2	3
S. D. of F_2 hoodeds	.90	.87
S. D. of uncrossed hoodeds	.38	.47
S. D. of uncrossed hoodeds as published....	(.75)	(.53)
Increased by cross	.52	.40

If the difference between these selected generations lies in the changed position of the mode of continuous ger-

minal fluctuations, one would have difficulty in accounting
for the above facts.   If these various selected generations
differ in the number of multiple factors they bear, one
can easily understand that the reason that practically no
modification is apparent when the third generation is
crossed, is that the number of plus factors in this genera-
tion and in the wild are not very different; in the fifth
and sixth generations there may be a few more plus fac-
tors than in the wild, and in the tenth generation there
are several more.

.13. The early generations of the plus race, although
only very slightly lowered by crosses with wild, are strik-
ingly lowered by crosses with Irish.   In a cross in which
the hooded parent came from the second generation, the
lowering was .83; when the hooded parent crossed came
from the third generation, the lowering was 1.65.   Now
how may this fact be interpreted?   If the change in the
means following a cross be assumed to be due to the ac-
tion of different numbers of factors in the races crossed,
it is clear that this particular wild is more like the plus
race in regard to its factors than is the particular Irish
race.   In other words the wild race seems to have more
plus factors than the Irish race.   When early generations
of the plus race are crossed with wild there is hardly any
change in the averages of the F_2 hoodeds, because there
are about the same plus factors in the wild as in these
early generations of the plus race.   When these same
generations of the plus race are crossed with Irish there
is a considerable decrease in the averages because there
are fewer plus factors in the Irish than in the early gen-
erations of the plus race.   Now if the germ plasms of the
wild and Irish differ in regard to the number of accessory
factors, and if the germ plasms of the plus and minus
races differ in this same regard, comparisons of all the
crosses between these races should show the following
results: crosses between wild and minus should give
greater modifications in F_2 than crosses between wild and
plus; crosses between Irish and minus should modify the

F_2 hoodeds less than crosses between Irish and plus. More directly, the plus race should be more modified by the Irish, the minus race more modified by the wild. Observation of Table I will show that these results are realized.

As already noted in the case of crosses between the plus and wild races, this table shows that in other crosses the different generations of the selected races are differently modified. After long selection there is more modification as the result of crossing. This generalization is supported by all the averages and all the standard deviations in crosses involving the wild race; it is supported by all but one average and by all but one standard deviation in crosses involving the Irish race. If selection is sorting out different groups of factors in the plus and minus races, crosses made after many selections bring together groups of factors more diverse than when crosses are made after only a few selections. The greater the diversity in the numbers of plus or minus factors in the animals crossed, the more extensive will be the segregation in the second generation. More extended segregation may be expressed by increased variability and by more pronounced modification of the averages of the F_2 hoodeds.

14. The behavior of the ''mutant'' in crosses with the plus and minus races gives clear support to the multiple factor hypothesis. Castle (Castle and Phillips, :14, p. 29) has clearly demonstrated this point. The ''mutant'' is a suddenly appearing, quantitatively increased stage of the hooded character, that is controlled by a Mendelian factor. Crossed with the race from which it sprang, the extracted individuals show no change from the uncrossed race, either as to averages or variability; crossed with the other race, modifications were found, equalling those obtained when the two races were crossed together. The newly discovered factor acts independently of the other factors, is not modified by them, and does not modify them. Being the one difference between

the mutant and the plus race at the time the mutant appeared, this factor affords a critical test for the interpretation of the modifications that result from crosses.

OBJECTION TO THE MULTIPLE FACTOR INTERPRETATION

One new point since 1914 has been urged against the application of the multiple factor hypothesis to the results. By the strength of this evidence the authors of the rat publication are "forced to conclude that this unit (hoodedness) itself changes under repeated selection *in the direction of selection* "; (Castle, :15*b*, p. 722). The point follows:

> The changes effected by selection show permanency under crosses with wild rats. They change no more nor less than an unselected hooded race does. A first cross of the selected race seems to show a partial undoing of the changes produced by selection, but a second cross made on a still larger scale, involving over 1,000 second-generation individuals, showed no further change of this sort, but instead a return to about what the selected race would have been had no crossing at all occurred (Castle, :16, p. 96).

If the grade of hooding of the plus race is reduced in crosses with wild by the replacement of factors selected out of the plus race, repeated crossing of the modified rats should produce further reduction. On the basis of the above claim that crosses do not produce such modifications in the hooded pattern all the evidence formerly admitted to favor the multiple factor interpretation has been swept aside. No one would claim that a single strongly supported experiment may not upset large amounts of contrary evidence, but in such cases it is of utmost importance to have the validity of the crucial experiment fully supported. Is the claim that crosses do not change the selected races fully supported? The following are all the data we are given on this point:

Extracted hoodeds from
    hooded × wild .... 75 rats, average 2.89; regression on grandparents .56
Extracted hoodeds from extracted
    hooded × wild .... 263 rats, average 3.33; advance on grandparents .32

Averaging the 75 hoodeds may first be criticized. These include all the extracted hoodeds that came from crosses between the wild and the plus races. The third, fifth, sixth and tenth generations of the plus race are involved. It has been shown that the early generations of the plus race are not lowered very much by the crosses in comparison with the tenth generation, which was considerably modified. Therefore among these 75 extracted hooded rats are some that were lowered by the crosses, but more that were practically unmodified. Moreover, the 263 twice extracted hooded rats came from ancestors that had been selected for at least ten generations. Only 16 of the 75 once extracted rats had ancestors that had been selected for ten generations; the others, having ancestors selected for a shorter time, would be expected to give lower averages. In testing for further lowering in this second cross it would seem unjustified to use an average including rats not lowered by the first cross or rats that had not been selected for an equal number of generations before the crosses. Modified by the above considerations the comparisons stand as follows:

Extracted hoodeds from
   tenth gen. plus × wild ....average 3.15
Extracted hoodeds from
   extracted hoodeds × wild ....average 3.33
Uncrossed, same generation and
   grade as hooded grandparent ....average 3.84

The conclusion has been quoted that the cross of the extracted hoodeds with wild has not carried on a further reduction, but it has shown a return, "to about what the selected race would have been had no crossing at all occurred." Will the above figures support this conclusion? The cross of the extracted hoodeds with wild does indeed give a higher F_2 average than the cross of the tenth generation, but the difference is only slight (.18). These two averages are based on very different numbers. It is entirely possible that a larger number of rats extracted from the first cross would have had a higher average than that of the rats extracted from the second cross; in such

a case the second cross would be said to show further reduction.

Whether this advance in the second cross returns the hooded grade to about what the uncrossed race would have been is a matter of what average is used to represent the uncrossed race. The original hooded parents were the last parents to be selected in this series of crosses. It seems clear then, as above reasoned in another connection, that the average to be used in comparison with the two groups of F_2 hoodeds is that of the *offspring* of uncrossed parents of the same grade and generation as the original hooded parents used in the crosses. If this average be accepted (3.84), it is plain that even after the second cross there remains a considerable difference between the averages of the uncrossed and the twice extracted hooded rats. There is reason to believe that the changes produced by selection *are* modified by crossing and that it has not been finally disproved that further crossing does not cause further modification. So, as far as can be judged from the data at hand, this crucial test does not seem to offer a final blow to the applicability of the hypothesis of multiple factors.

On the other hand, that modification actually does result from crosses is strikingly proved by the conversion of the minus race into the plus by means of a cross. This experiment has been referred to on page 729. Six successive return selections did not return the average of the minus race to the ''0'' grade. But after minus race rats were crossed with wild, a single selection of the plus varieties raised the average 2 grades above ''0.''

Summary

By way of recapitulation, the points referred to are summarized as follows:

A. Seventeen generations of selection need not have entirely eliminated modifiers, because,

    1. Matings less close than brother and sister have tended to continue heterozygosity;

2. Environmental influences may possibly act in such a way that only occasionally does a selected individual carry germ plasm more homozygous then the average.

B. The implied claim that the facts do not support the supposition that selection has decreased the number of modifiers, or has reduced the heterozygosity in the two races of rats, has been answered by the following points:

1. Selection reduces the variability.

2. The rate of advance declines as selection is continued.

3. Parental regression is lowered by selection.

4. Return selections argue that heterozygosis is still present; they indicate that there is less heterozygosis after longer selection, since selection reduces the effectiveness of return selections.

5. Crosses between the plus and minus races strongly suggest that heterozygosity is still present by the increase in variability in F_1; they also appear to show that there is less heterozygosity in a later generation, since the increase in F_1 is less in a cross after longer selection.

6. Crosses between the selected races and the wild or the Irish race show that more modification appears in the F_2 hoodeds when crosses are made after longer selecting.

The reader is now in a position to judge whether the writer is justified in concluding that there is still a "possibility that other as yet undiscovered factors might be responsible for the apparent changes observed" (Castle, :15, p. 722) and that the claim that "all the evidence we have thus far obtained indicates that outside modifiers will not account for the changes observed" is too sweeping.

Discussion[1]

A great difficulty has been placed on the discussions of this subject by the different terminology used by those holding different opinions.   Calling the visible character the Mendelian unit is a striking example of this difficulty. There is a vital difference between a unit character and a factor, which must be constantly recognized if this discussion is to progress.

It is unfortunate that the word *selection* has come to have the significance of a slogan.   For the nature of the actual power of selection itself is not in question.   What selection is, can be easily defined and agreed upon.   If the nature of the changes in the germ plasm could be determined, there would be little disagreement as to what selection could accomplish.   Even those who are not considered to be selectionists believe that natural selection is very important in evolution.   So the epithets, selectionist and pure-lineist, fail to indicate the difference between the two groups to which they have been applied.   It would be quite impossible to divide biologists into two distinct schools on the basis of a subject upon which there are many different shades of opinion.   Any such classification would be inaccurate, even if the most precise definitions of the classes were generally accepted.   When there are no accepted definitions, and those most clearly cut are offered by individuals in the opposite group (each one realizing the diverse ideas within his own group and wishing to crystallize an opposing view in order to attack it) such classification of opinion is far from scientific.   In the present instance, the classification into selectionists and pure-lineists has tended to magnify the differences between investigators.   With a desire to try to overcome

[1] Since the writing of this paper, there have appeared papers by Pearl ("Fecundity in The Domestic Fowl and The Selection Problem," Amer. Nat., 1916, p. 89) and Castle ("Can Selection cause Genetic Change?" Amer. Nat., 1916, p. 248) which have a close relationship to the present discussion. It has been considered wiser to leave this paper as written, than to enter the controversy by including discussions of the two papers mentioned.

the exaggerated differences which seem to exist, the following discussion is offered. It is written with no wish to codify or defend the opposing positions, but rather as an attempt to formulate the issue a little more clearly by presenting two views, which appear to have advocates, of the nature of the changes in the germ plasm.

The view to be called the "first" is as follows: The changes in the germ plasm are in the nature of fluctuations, now larger, now smaller, but continuously appearing; they lead in all directions. This is true of all inheritance, whether or not it be factorial (Mendelian) in basis. If it refers to Mendelian inheritance the potential grade of the factor in question, as found in any zygote, acts as a mode about which the fluctuations in potentiality occurring in the next generation are grounded. In other words, although a zygote may include the strongest potential grade of a factor that has appeared, the inevitable fluctuations in this factor that are found in the different gametes formed by this zygote will include, together with those like and weaker than the parent, some with stronger potentialities than the parent.

The view to be called the "second" is as follows: The changes in the germ plasm are discontinuous; they appear fortuitously. They may strike out in almost any direction, as a projectile may be aimed in "any direction," in contrast to the "all directions" taken by the waves of sound when the projectile explodes.

According to the first view, selection would result in modification in any direction the breeder might desire, irrespective of variational tendencies shown by the animal. To maintain conformity to type would require as constant selecting as would be required to obtain divergence. According to the second view, selection could progress only in certain directions, depending on how the germ plasm happened to change; the variational tendencies of the animals would probably suggest these directions. Conformity to type would be considered to be a fundamental phenomenon due to the conservative tend-

ency of the germ plasm to maintain the *status quo*.   On the basis of the first view, the external influence (selection) would have major importance in defining the course of evolution; on the second, the internal influence (the inherent nature of the germ plasm itself) would have major importance.   In both cases, the nature of the progeny would depend on the nature of the germ plasm of the parents.   In both cases selection would be able to modify the race.   But in neither case is the origin of the changes in the germ plasm explained.   The fundamental causes of evolution are as much a mystery as ever. Grant a certain hypothesis of germinal changes, and selection becomes a more important factor in evolution than when another hypothesis is granted.   But even such an increased importance of selection does not give it the value of a fundamental creative cause of evolution.

There has appeared a theory that would give selection still greater importance by saying that selection has the power to build up unit-factors and induce mutation.

Unit-characters may arise gradually as the result of repeated selection in a particular direction (Castle, :12*b*, p. 280).

In yellow animals, as in blacks, individuals of varing intensity occur the darkest known as reds, the lightest as creams.   A complete series of intermediates can be obtained if so desired.   If we select any two widely separated stages in this series fairly stable in their breeding capacity and cross these, they Mendelize, *i.e.*, they behave as if they were a single unit-character difference between them. . . . That difference might equally well be *half* as great as it is, or a *quarter* as great, or a thousandth part as great.   A monohybrid ratio would result equally in each case, upon crossing the two quantitatively different stages (Castle, :12*a*, p. 358).

Now this may be true for yellow guinea pigs, but the rats clearly demonstrate that it is not true in all cases. The two quantitatively different stages of the hooded pattern represented by the plus and minus races do not result in a monohybrid ratio when they are crossed.

However there has appeared a ''unit-character'' difference in one of these races of hooded rats.   It appeared suddenly, and it Mendelizes when crossed with other

hooded rats. The occurrence of this "mutant" is claimed to have been induced by selection.

It seems to us quite improbable that this plus mutation could have arisen in the minus selection series. We believe that the repeated selection which was practiced had something to do with inducing this change in the plus direction (Castle and Phillips, :14, p. 31).

No reason for such a supposition is given. On the other hand there is clear reason for supposing that such a mutation would be far more easily detected in the plus series if it occurred there. The same mutation occurring in the minus race would perhaps have the same relation to that race as it had to the plus race when it occurred there; since it would lack the extension factors of the plus race, it would have a very different appearance and would probably have a grade not far from "0." It seems that very few rats of this grade were bred or tested. Had this mutation occurred in the minus race and been isolated, it would have been possible to obtain it as it appeared in the plus race, by proper crossing.

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SOME FEATURES OF ORNAMENTATION IN THE KILLIFISHES OR TOOTHED MINNOWS

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THE killifishes, so named by the early Dutch settlers about New York from their habit of living in the channels or kills, embrace an interesting family of fishes. They are known by other names, as top-minnows, cyprinodonts, toothed minnows, millions fish, etc. Some of these names are, however, more limited in scope and pertain to sections or genera. Top-minnow was applied from the habit of many living at the surface, and cyprinodont, meaning toothed carp, arose as some greatly resemble very small carps or true minnows (Cyprinidæ), though were found to differ in the presence of teeth in their jaws. Besides this character are a number of others, in which they agree with several related families to form the order of pike-like fishes (Haplomi). Such are all internal and largely have reference to the bony skeleton. In the abdominal ventral fins (*Procatopus* excepted), and without true spines in the dorsal and anal fins, the order resembles the herring-like fishes (Isospondyli), but differs in the absence of a mesacoracoid bone. This latter character is in agreement with the host of spiny-rayed fishes (Acanthopteri), but they usually have the ventral fins well anterior.

Though six families are included in the order of pike-like fishes, only the mud-minnows (Umbridæ) and the pikes (Esocidæ) occur in the Middle Atlantic States. The killifishes differ from both in the extremely protractile premaxillary bones, a condition very easily demonstrated by examining the upper jaw and prodding its edge forward. In form the body is oblong from elongate and slender to deep and nearly orbicular. The head is usually large and robust, often quite chunky. The mouth is small, with short gape, though wide and terminal. The teeth are extremely diverse, from broadly incisor-like to finely

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villiform, and usually occur only in the jaws. The pharyngeal bones, unlike those of the true minnows or cyprinoids, often have fine teeth, rarely molar, and never modified or in even numbers as in cyprinoids. The scales are mostly large, cycloid, adherent, regular and without a perfected lateral line. The dorsal and anal fins are single, inserted usually behind the middle of the body, but no adipose fin developed. The caudal is broad and, though sometimes pointed, not forked. The paired fins are placed low, and the ventrals abdominal.

Many genera and species, about sixty belonging to the first and over three hundred to the last, have been described. Of these about ten genera and fifty species occur in the United States. The family reaches its greatest diversity in tropical America, and in the Old World the largest number of forms occur in African fresh waters. Killifishes live in fresh waters in nearly all situations, in lakes of great elevation, or in sandy desert streams, puddles and ponds. Others live in tidal waters, or along the shores of sea-beaches, and all near or close to the surface. The great changes with age, sex and season render many of the species difficult of determination. All are of small size, less than a foot in length.

In nearly all killifishes the sexual differences are well marked, at least during the spawning or breeding season. Often the males have enlarged fins, smaller in the females, as in the may-fish (*Fundulus majalis*) and the zebra-fish (*Fundulus zebrinus*). Still other characters occur in some species which have been entirely overlooked or scarcely noticed by most writers. These are the minute spines, or spinules, adorning the scales and fin-rays of certain species during the spawning-season. Garman, in his celebrated monograph of the killifishes,[1] simply says, "a minor sexual character is that of small spines appearing on the fins of males in several genera in the breeding time." I have been unable to find any detailed account of these structures, except casual reference to a few in descriptions of species. These are usually quite short and

[1] *Mem. Mus. Comp. Zool.*, XIX, 1895, p. 11.

of but slight value. So far as I have been able to examine material, these little spines occur only in certain species of the true killifishes, the pursy-minnows and the four-eyed fishes, or the Fundulinæ, Cyprinodontinæ and the Anablepinæ, respectively. I have never seen any in the top-minnows. It is interesting to note that the four-eyed fishes, creatures with remarkable and extreme modifications of structure, should be the only group of viviparous forms in which the spinules have so far been found to occur. These spinules are different in several ways from the nuptial tubercles of cyprinoids, in that they are more permanent, though very minute and inconspicuous. They may easily be overlooked in preserved examples, owing to the mucus exuded and covering the scales and fins. This should be carefully cleaned away, before they can be detected, and even then only with a good lens. Each spinule is found to arise on or close to the edge of the scale, and not on its exposed surface, as the more distinctly straight conic tubercles of the cyprinoids. The spinules are not always perfectly firm and rigid, but may be flexible or delicate. Those on the anal fin rays are generally curved slightly and are also often close together, though not perfectly regular. Their arrangement or design is usually more or less complete in each species. At least in one species their development occurs in the young, as in the ornatus stage of the common mummichog. Probably the spinules in most species are not permanent, but disappear after the spawning-season. However, if the spawning-season for a certain species is protracted, males with spinules may be found for a period of several months. Preserved specimens of killifishes do not show scars or pits like cyprinoids, and it may be that the spinules wear away as well as drop off. I have not found any examples with spinules in cold weather, or when spawning was apparently over. In no case have the inner edges of the pectoral rays been found with spinules, like the tubercles of certain cyprinoids. Doubtless such developments are to be correlated with the spawning habits, as none of the

EXPLANATION OF FIGURES

All the figures are drawn to the scale of millimeters and the accompanying numbers signify such, so that the number of times the line is contained in the lengthwise diameter of the figure will give its dimensions.
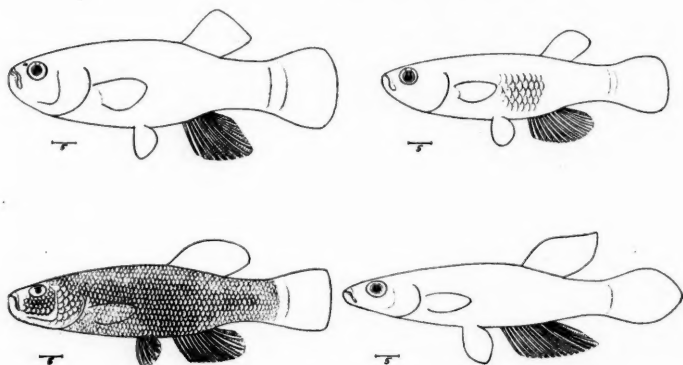
PLATE 1

Fundulus nisorius Cope.   *Fundulus floripinnis* (Cope).
Fundulus zebrinus Jordan and Gilbert.   *Fundulus stellifer* (Jordan).
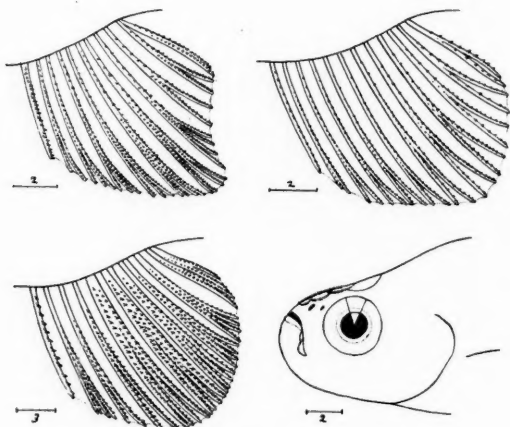
PLATE 2

Fundulus heteroclitus macrolepidotus   *Fundulus diaphanus* (Le Sueur).
   (Walbaum).   *Lucania parva* (Baird).
Cyprinodon bovinus Baird and Girard.
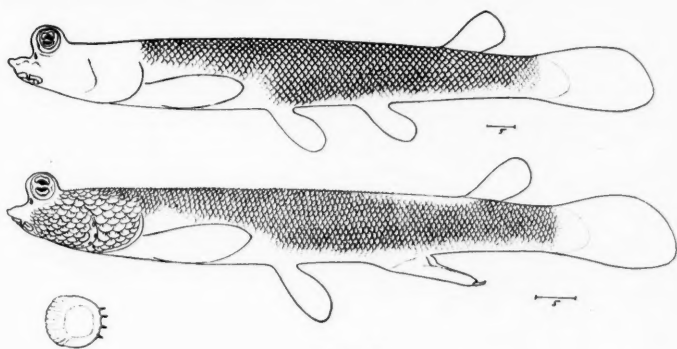
PLATE 3

Anableps anableps (Linné).   Upper figure female, lower male, and enlarged scale to left.

killifishes have been seen to clasp the female as Reighard describes the creek chub (*Semotilus atromaculatus*).   In the spawning behavior of the mummichog I could not determine if the male in any way secured or held fast to the female by means of his anal spinules, though possibly they may be of some such use.   Killifishes greatly parasitized with sporozoa or myxosporidia have been found, the adult spawning-males sometimes greatly distorted, though with the development of the spinules more or less perfected.   Among species of killifishes represented by spawning-males without spinescent ornamentation which I have examined are *Fundulus punctatus, F. similis, F. majalis, F. luciæ, F. nottii* and *F. notatus*.

In the common killifish or mummichog (*Fundulus heteroclitus macrolepidotus*) of the tidal waters of our Atlantic coast, the male is furnished with little spinules on the anal rays.   They are better developed on the outer or terminal branches of the rays.   They are also often irregularly placed, though usually a pair may be found on each segment, or as a spinule projecting out on each side of the fin.   None of the scales or other fins with spinules.   Spawning-males 76 to 82 mm. long.   The female has a well-developed anal tube extending along the front of the anal fin for at least half the length of the depressed fin.

My examples 92 to 96 mm. Spawning fishes of this species were obtained from April until the middle of August.

In the West African killifish (*Fundulus nisorius*) the male has the outer portions of the anal rays covered with little spinules. It is also quite likely that the anal fin is furnished with spinules in the spawning *Fundulus bermudæ*.

The barred killifish (*Fundulus diaphanus*) common in the fresh waters of the east, from Maine to Carolina, is quite brilliant in the spawning-season. In the male the spinules are arranged as little points, like those of the mummichog, though as the fish is smaller they are less conspicuous. The scales and fins other than the anal are without spinules. Spawning males 60 to 70 mm. In the female a well-developed basal anal sheath extends around the front of the anal fin. Spawners of this species in full color were obtained from April until the middle of August.

The zebra-fish (*Fundulus zebrinus*) of the Mississippi Valley region has long been noted for its prickly appearance. Jordan and Evermann state, presumably with reference to spawning fish, " in males the margins of both dorsal and anal fins are evenly rounded, the anal the higher, its rays beset with minute white prickles." My examples show it differs from any of the preceding species in the male having the sides with the scales minutely spinescent along their edges. The area of spinescent scales extends from the head in some examples, in others for variable distances, back to caudal base, and always with its greatest development over the base of the anal fin. On the back the spinules gradually disappear, and the same is true on the under surface of the caudal peduncle. Further, an additional modification is seen in the presence of spinules on the inner or hind surfaces of the ventrals, though these fewer than on the anal rays. On the front of the anal fin the spinules are best developed, though irregularly distributed on the segments of the fin-rays, here and there appearing crowded or sparse.

Length 48 to 75 mm. The female has a broad basal sheath around the front of the anal fin. Length 51 to 63 mm.

The little green killifish (*Fundulus floripinnis*) of the South Platte River basin has the male with the scales along the middle of the side, especially above the base of the anal, with minute prickles along their edges. Similar prickles also occur on the rays of the anal fin, though with irregular distribution on the segments. They usually appear better developed along the front anal edge. I have also seen a few minute prickles above the eyes. In length these males were 47 to 57 mm. This species belongs to the section *Zygonectes* Agassiz, so called as the fishes were said to swim in pairs. Doubtless this would refer to the spawning-habits or when spawning, for at other times they do not appear to swim in pairs. As in the brown killifish (*Fundulus luciæ*), another member of the *Zygonectes* group, I have never seen them swimming in pairs, and Ellis claims the same for the little green killifish.

In the stud-fish (*Fundulus stellifer*) the males have very minute spinules along the anal rays and along the edges of their scales above the fin. They are also irregularly placed. Length 82 to 99 mm., and the females 73 mm. long have a well-developed basal anal sheath at the front of the fin. The related *Fundulus catenatus* shows similar ornamentation in the male, though my material is inadequate for detailed comparison.

In the rainwater-fish (*Lucania parva*) males in high color, taken in June, differ from any other killifish I have examined in the presence of minute spinules on the upper surface of the snout, in some cases even encroaching on the interorbital space. No other spinules occur. The muzzle of the male is also modified or decidedly obtuse, suggestive of the fat-head minnows (Pimephales).

The pursy-minnows (*Cyprinodon variegatus*) when in brilliant spawning-dress, in the case of the males, are extensively provided with minute spinules. These extend all along the edges of the scales on the head, front predor-

sal region, posterior sides of trunk or above anal fin, and front side of caudal peduncle. All the anal rays are also minutely and finely spinescent, though I have not found any spinules on the paired fins. Spawning-males 54 to 57 mm., and the females smaller. The related *Cyprinodon bovinus* of the southwest is similar. *Jordanella floridæ* is represented only by one small example with spinules, these very minute along the edges of the scales above the anal. No spinules found on any of its fins.

In the four-eyed fish (*Anableps anableps*) of South America, the males have an intromittent organ dextral or sinistral. They also have the scales on the trunk, especially above the anal and on the predorsal region, with spinules, though more numerous or dense with spinules in the former space. Top of head, belly and lower surface smooth. Sides of caudal peduncle with a few scattered spinules. A large female, 244 mm. long, is largely spinescent on the trunk above, though the spinules not so dense as on scales above the anal in the male. In females of smaller size, 124 to 128 mm. long, the spinules are rather obsolete, sparse and scattered, also only on the back and sides above. Young 27 mm. long still show the umbilical sac well developed and are scaleless.

Though I have not examined spawning examples of small-finned killifish (*Fundulus parvipinnis*), Jordan and Gilbert state, "scales large; in the males in spring roughened or ctenoid by small granulations and prickles, similar to the nuptial excrescences of some Cyprinidæ; fins also rough."

SHORTER ARTICLES AND DISCUSSION

FURTHER REMARKS ON THE INHERITANCE OF CONGENITAL CATARACT

AN article[1] by the writers in a previous number of this publication dealing with the inheritance of congenital cataract in a statistical way has been rather severely arraigned by Danforth[2] in a more recent issue. In our original paper we presented data taken from genealogical tables published by Harman in the "Treasury of Human Inheritance" which led us to believe—

1. That congenital cataract could no longer be considered as a single, dominant, unit character.

2. That Davenport should be criticized for making eugenical recommendations based on the inheritance of cataract as a dominant character when the method of inheritance is not positively known.

3. That from the evidence at hand cataract could better be considered as a single, recessive, unit character, reserving final decision as to this point until more complete data should become available.

Danforth believes with us that congenital cataract can not be considered as a simple, dominant, character. Nevertheless he tries to defend Davenport from "unjust" criticism while he does not agree with him. It is upon the assumption of cataract as a positive or dominant character that Davenport bases his eugenical recommendation as follows:

The usual method of inheritance is that of a positive character. Affected individuals have either half or all of their offspring affected, while two unaffected parents will probably not have defective offspring. However, as cataract usually appears late in life it is not always possible to predict whether the parent will become affected or not.

The eugenic rule is this: If either parent has cataract at least half of the offspring will have it also. If a person belongs to a strain that has cataract but is free from it, advice must depend on the nature of the cataract. If in the family strain cataract appears early, before the age

[1] Jones, D. F., and Mason, S. L., "Inheritance of Congenital Cataract," THE AMERICAN NATURALIST, 50: 119–126, February, 1916.

[2] Danforth, C. H., "Inheritance of Congenital Cataract," THE AMERICAN NATURALIST, 50: 442–448, July, 1916.

of the person who contemplates marriage, then such marriage may be advised; . . .[3]

As regards congenital cataract, then, Davenport advises that unaffected persons from affected stock can marry without fear of producing affected children. Harman's tables show over thirty matings of unaffected parents having at least one affected child.

No matter how unsatisfactory is the proof that cataract is a simple recessive, it should be borne in mind that the data given in Harman's tables do not stand the test when cataract is considered as a simple, dominant character. If the argument that heterozygous individuals sometimes show the recessive character is to be used to prove the dominance of cataract, it would be necessary to use the assumption to explain thirty-one exceptional families which have from one to eleven children of which 40 per cent. of the total are affected. On the recessive hypothesis there is only one exceptional family so far known to be explained. As long as it is not a simple dominant character it makes no difference whether it is a simple or complex recessive or a dominant governed by multiple factors, the eugenical recommendation quoted above should not be made, and we still believe that Davenport can be justly criticized.

Danforth objects to the disagreement between the observed and the expected results in our table I, giving the progenies of matings of normal by normal, and compares the goodness of fit unfavorably with data given by Usher on retinitis pigmentosa. In our results the disagreement lies in an excess of the actual number of affected children over the expected number. If, as Danforth says, " a certain number of congenital cataracts are produced by intrauterine poisoning without necessarily any reference to heredity " the tendency would be to raise the actual number of affected children above the expected. Also any cases of *origin de novo*, to which he believes we did not give enough consideration, would tend to have the same effect. Moreover, it should be noticed that Usher has over twice as many individuals to base his ratio upon, 320 as compared to 153 in our case.

Danforth states two main conditions which he says our assumption of cataract as a recessive character does not meet. The first is the low probability of an individual carrying the abnor-

[3] Davenport, C. B., ''Heredity in Relation to Eugenics.'' Henry Holt and Co., New York, 1911, pp. 111–112.

mality in a haploid or a diploid state meeting with a heterozygous normal, in random mating, which would be necessary to produce affected children. According to Danforth's calculations the probability of heterozygotes in the general population is one in thirty. He shows that Harman's tables give in some cases as high as eight out of nine individuals mating with normals and producing affected children, thereby showing that the normals are heterozygous on the recessive hypothesis. In the previous publication we did not give consideration to this point which is of noteworthy significance and we are indebted to the writer for calling our attention to it.

This apparently high proportion of heterozygotes in the general population would be a serious objection to our simple recessive hypothesis if it were not for the fact that there is a considerable amount of consanguinity recorded in the pedigrees given by Harman. With each of the pedigrees including from one to many families there is a definite statement as to whether a record was made and, if so, whether or not consanguinity was present. Tabulating these statements shows that in sixty of the pedigrees no record was made. In twenty-four no consanguinity and in eleven consanguinity was definitely recorded. Then in those cases in which a record was made nearly 50 per cent. of the pedigrees show more or less intermarrying. Altogether there are seventeen cousin marriages.

With this amount of intermarrying among affected stocks the proportion of heterozygous individuals carrying the abnormality in a simplex condition would be greatly increased over the proportion in the general population, and Danforth's most serious objection to our hypothesis loses its force. Evidently no consideration was given to this point when he says '' a more striking refutation of the assumption could hardly be found '' (p. 447).

With regard to the second main condition which is raised against the assumption of cataract as a single, recessive, unit character Danforth seems to be partly in error, if we understand his statement correctly. He states: '' If congenital cataract were recessive the normal children of a cataractous parent should themselves produce affected children in half as many cases as do their cataractous sibs, and the total number of affected children produced should be one half as great in the first case as in the second '' (p. 446). Since on the recessive hypothesis only heterozygous normal and homozygous abnormal sibs are produced

in equal numbers from matings of Nn $\times$ nn included in category B, and neither can produce affected children in turn, unless mated to a heterozygous normal or a recessive, we do not see why the normals should produce affected children in *half as many cases* as their cataractous sibs. If the chances for obtaining such mates were the same the number of matings which produce affected children should be approximately the same.[4]

The second part of the quotation is correct only when both normal and abnormal F_1 individuals have an equal chance to mate with individuals who are either affected or carry the abnormality in a recessive condition. The chances of the two classes mating with such individuals are probably not equal because individuals affected with cataract would have a harder time to find a mate than their normal brothers or sisters and there would be a greater tendency towards consanguineous marriages and consequently a greater chance of mating with cataractous individuals. The frequent intermarrying among affected stocks is well known with other abnormalities. Hence normal persons carrying the affectation in a heterozygous condition would be more likely to marry into unrelated stocks with a far less proportion of heterozygous individuals than would their affected sibs. If this is true then the expectation of the number of matings of affected F_1 individuals giving affected children in turn

[4] In answer to a letter sent to Dr. Danforth asking about the above point the following was received which shows that we did not understand his meaning correctly:

In reply to your letter of September 29, I do not say in the paragraph to which you refer that of the children of cataractous parents half as many normal as cataractous *individuals* should produce affected offspring but, on the contrary, that the normals, taken as a group, should produce affected children ''in half as many cases'' (*i. e., at half as many births*) as do the cataractous. The families of *numerous individuals* in both groups would be expected to contain no cataractous individuals at all, but in those families (equal number for each group) where such children may occur there should be in the long run half as many cases in families with normal parents as in families with one cataractous parent. The remainder of the paragraph to which you refer and the statements in your letter show, I think, that we are in complete agreement as to the theoretical expectations; it was my unfortunate use of the word ''cases'' which no doubt caused you to raise the question. I meant it to refer to F_3, you doubtless suspected it might refer to F_2. This suspicion was undoubtedly strengthened by my ''relation of one to two'' which is one of those slips of the pen for which I have no means of accounting. Of course it should have been ''one to one'' and that fact was uppermost in my mind at the time of writing the passage!

would be greater than that of the unaffected and the total number of children would therefore be more than twice as great.

Danforth, however, after raising this condition does not determine the number of affected children from the affected and unaffected F_1 individuals, but calculates the percentages of these two classes of parents which produce at least one affected child. He finds that eighty-six per cent. of the cataractous children of a cataractous parent themselves produce some affected children and thus presumably have mated with heterozygous normals. Of the normal children from the same F_1 generation only ten per cent. produce affected children. If the chances for securing similar mates were the same these percentages should be approximately equal. The relation of ten to eighty-six which does not conform to a one to two ratio as Danforth states that it should necessarily deviates still more widely from a one to one ratio.

There are two reasons why this deviation from a one to one ratio can be expected in favor of a larger number of affected matings giving affected children than of unaffected matings. The first lies in the fact that matings of affected by heterozygous normals should give a one to one ratio of affected and normal children, whereas the matings of heterozygote by heterozygote should give a ratio of one to three. As was emphasized in our previous publication the only criterion by which it can be determined whether the mates to the two kinds of F_1 individuals are heterozygotes or homozygotes is the production of at least one affected child. In families with a small number of children the matings which promise a one to one ratio would have a greater chance of producing at least one affected child than matings which promise a ratio of one affected to three unaffected children. Hence more of the families of the latter than of the former class would be omitted from the data.

The other reason why the deviation that Danforth obtains can be expected is that which has already been mentioned, namely, that affected individuals are more likely to marry related individuals because of the greater difficulty of obtaining a mate than the unaffected would have. The proportion of heterozygotes in affected strains would be far higher than in the general population, so that the chances of the two kinds of F_1 individuals mating with a heterozygous normal would not be equal as Danforth considers them to be.

It is recognized that these arguments are extremely indefinite and that it is difficult to determine just how much value to give

them.   They are however hardly necessary since the numbers ninety-six and forty-seven upon which Danforth bases his criticism are too small to make a really critical comparison.

Since the number of affected F_1 individuals which should give one half affected children exceeds the number of unaffected F_1 individuals which should have only one third affected children, the actual number of affected children in the two kinds of F_2 populations would deviate proportionally farther from a ratio of one to two.   If it is conceded that the chances for the two kinds of matings are not equal, then this deviation would be expected.

The three cases in category C which we gave as matings of abnormal by abnormal which theoretically should give only abnormal children according to the simple recessive hypothesis can be found in Harman's tables in the "Treasury of Human Inheritance"[5] as follows: Table 309, Parents I, 1 and 2—Children II, 1 to 5; Table 312, Parents II, 3 and 5—Children III, 3 to 4; and Table 342, Parents III, 28 and 37—Children IV, 60 to 66. Danforth says that he can find only two of these.   They are probably 309 and 342.   The one which occurs in Table 312 should not have been used without an explanation.   Although the chart indicates that both parents are affected as well as their two children, the description of the family shows that the exact parentage is somewhat in doubt.   It was an error on our part not to mention this fact.

With regard to the family in 342 in which part of the children are normal where only abnormals are expected, Danforth does not accept our explanation that heterozygotes sometimes have the recessive character.   This is quite frequently shown in other material.   His refusal to accept this explanation to account for the one exception to the recessive hypothesis is shown in the following quotation: "a single bona fide case in which two affected individuals produce normal offspring is sufficient to overthrow it" (the recessive hypothesis) (p. 447).   We can not understand his refusal to accept this explanation to account for one exception when he is willing to use it to explain thirty-one exceptions to the dominant hypothesis!   This is evident from the following quotation previously alluded to:

Again, since Jones and Mason elsewhere in the same paper (p. 124)

[5] Harman, N. B., "Treasury of Human Inheritance," Eugenic Laboratory Memoirs, XI, Part 4, Section XIIIa, pp. 126–169, Pl. XXVIII–XXXIII, Dulau and Co., London, 1910.

use the same argument that "heterozygous individuals sometimes show the recessive character," we might, if necessary, use the same argument to prove the dominance of cataract. On the assumption that congenital cataract is dominant instead of recessive it might be maintained that in those cases where both parents of affected individuals seem to be normal, one of them is, after all, heterozygous—and affected children are therefore to be expected (p. 444).

Perhaps Danforth would be willing to consider another explanation which he suggests, that somatic cataracts of a congenital origin are not uncommon. If one of the parents in question had a somatic cataract the appearance of normal children would be expected but not of affected children unless the parent was also heterozygous for hereditary cataract. A probability which would be rather remote but not impossible.

From the data as they have been gathered up to this time it seems impossible to arrive at an explanation of the mode of inheritance of cataract which will be entirely satisfactory. While more proof is awaited, we believe that the assumption of congenital cataract as a single, recessive, unit character has the best support from the facts at hand. The article by Danforth has brought out several important considerations which we neglected. It is regretted that in this paper which at first sight makes out a strong case against our recessive hypothesis there is nothing offered towards a different solution of the problem.

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THE STATUS OF FOWLER'S TOAD, *BUFO FOWLERI* PUTNAM

S. P. Fowler, of Danvers, Essex County, Massachusetts, appears to have been the first to recognize the fact that this toad differed in many respects from the common toad. In a letter[1] to Prof. F. W. Putnam, Fowler gave a very accurate and complete account of the song and habits of this toad as he had observed it around Danvers.

Cope (see loc. cit.) discussed in much detail *Bufo lentiginosus fowleri* (Putnam). Little was known of this toad at the time Cope wrote. In fact, Cope stated that it was confined to a few

[1] Cope, E. D., "The Batrachia of North America," Bull. 34, U. S. National Museum, 1889, pp. 279–281.

ponds in northeastern Massachusetts, near the town of Danvers. He says:

Such a limited distribution for a land vertebrate is remarkable, as is also the fact of its having so long remained without introduction to science.

Cope's work was published in 1889, in the same year that Allen[2] reports having heard Fowler's toad in New Hampshire. Speaking of *Bufo americanus* Le Conte, Allen said:

After the breeding season, the toad's song changes from a prolonged pipe to a shorter, lower-toned note, that, at night, has a peculiar weirdness, and almost reaches a wail.

Although Allen thought that the common toad was responsible for the two songs, it is plain that he had heard the unmistakable song of Fowler's toad. Allen's observation extended the range of this toad well up into New Hampshire.

Although as late as 1889 Fowler's toad appeared to have a very local distribution in New England, more recent work has shown that this toad has an extended range southward.

In 1907 the writer[3] published a paper showing that Fowler's toad is very common around Oxford and Worcester, in Worcester County, Massachusetts. In a second paper, published in 1908,[4] it was shown that the range of this toad extended through Washington, D. C., and Chapel Hill, North Carolina, into northern Georgia, where it appeared to be the only common form in the vicinity of Hoschton and Thompson's Mills, near Gainesville.

In 1910 Miller and Chapin[5] gave an excellent discussion of the range of *Bufo americanus* and *Bufo fowleri* in New Jersey and adjacent regions of New York.

From the observations of Miller and Chapin it appears that Fowler's toad occupies practically the entire state of New Jersey, except, perhaps, the extreme northwestern part. Throughout

[2] Allen, Glover M., ''Notes on the Reptiles and Amphibians of Intervale, New Hampshire,'' *Proc. of the Boston Society of Nat. History*, Vol. 29, No. 3, 1889, p. 71.

[3] Allard, H. A., ''Fowler's Toad, *Bufo fowleri* Putnam,'' *Science*, N. S., Vol. 26, No. 664, Sept. 20, 1907, pp. 383–384.

[4] Allard, H. A., ''*Bufo fowleri* in Northern Georgia,'' *Science*, N. S., Vol. 28, No. 723, Nov. 6, 1908, pp. 655–656.

[5] Miller, W. De W., and Chapin, James, ''The Toads of the Northeastern United States,'' *Science*, N. S., Vol. 32, No. 818, Sept. 2, 1910, pp. 315–317.

central and southern New Jersey it is the only species, as *B. americanus* was not found here.   Miller and Chapin also found that Fowler's toad was the only form to be found upon Staten Island, N. Y., as well as upon Long Island.   In the mountainous parts of northern New Jersey both *B. americanus* and *B. fowleri* occur.

In 1914 Overton[6] published an interesting paper concerning the frogs and toads of Long Island.   Overton found that *Bufo fowleri* is the only toad occurring on Long Island, where it appears to be common, while the common toad of the mainland of New York State is *B. americanus.*

Various authors have mentioned the song of *Bufo fowleri.* S. P. Fowler in the letter to Professor S. W. Putnam, previously cited, first described its song.   His description is particularly apt.

To my ears the croak is a sharp, disagreeable, unearthly screech, difficult to describe, as it is unlike any sound I have ever heard.   A chorus of these has been likened to the whoop of a party of Indians.

As none of us at this late day can recall the whoop of Indians, this comparison, although historically interesting, does not give us much aid in appreciating the peculiar nature of the sound.

Dr. Nichols, in the same letter, is cited as considering the song to be a shrill monotone in a high falsetto voice, longer and more trilling than the voice of Pickering's *hyla.*   Fowler, however, states that there is no trill to the note, an opinion the writer also shares.

The writer has described the note as follows: "I have heard nothing in nature so weird and unearthly as the almost agonized wail of this toad, repeated at intervals,"[7] and "The usual note of Fowler's toad is a brief, penetrating, droning scream."[8]

Miller and Chapin, in their article previously cited, say of it:

. . . it certainly has much less music to it than the trill of the American toad.   The notes are more closely connected, so that a sort of buzzing is heard.

Miss Dickerson[9] says of the notes of *Bufo fowleri:*

6 Overton, Frank, "The Frogs and Toads," Long Island Fauna and Flora, III.   In the Museum of the Brooklyn Institute of Arts and Sciences, *Science Bulletin,* Vol. 2, No. 3, Nov. 3, 1914.

7 *Science,* N. S., Vol. 26, No. 664, Sept. 20, 1907.

8 *Science,* N. S., Vol. 28, No. 723, Nov. 6, 1908.

9 Dickerson, Mary C., "The Frog Book," 1906.

The call of the Fowler's toad is a metallic, droning sound, not conspicuously vibrated. The pitch of the call may be as high as that of *Bufo americanus,* but descends in doleful fashion through several intervals before the close. Its carrying power is unusually great. The quality is indescribable; on the whole, the call is weird and mournful and not especially agreeable to our ears.

Overton (previously cited) says:

Its song is a combination of a low whistle and a moan, and the two sounds do not melt into a chord. The combined sound is discordant and decidedly unpleasant to a musical ear, but at a distance the sound is more pleasant for the moan is not apparent and only the whistle is heard. The sound lasts from two to three seconds and may be repeated at intervals of about ten seconds.

Overton says the song of *Bufo americanus* is prolonged about thirty seconds.

Dr. Andrew Nichols,[10] of Danvers, Massachusetts, is quoted as saying:

There is no sound in bog, pond, fen, forest, or air at all like it.

Although Nichols referred to the toad as *Bufo lentiginosus* Shaw, it is extremely probable that he had in mind *Bufo fowleri.*

Miss Hinckley[11] says:

The bleat of *B. fowleri,* with its far reaching, metallic ring, is usually heard after sunset. I have seen the latter give voice on the land, while the trill of *B. americanus,* heard at all times of day and night during the mating season, I have only seen given in the water.

In the field the writer has found little difficulty in recognizing Fowler's toad throughout its range. Its note at once distinguishes it from *B. americanus.* Color characters, while fairly definite, do not, perhaps, always serve to distinguish *B. fowleri* from *B. americanus.* According to Miller and Chapin, the color of the eye alone will distinguish *B. fowleri* from *B. americanus.* These observers state that in the former the iris is silvery, while in the latter it is bronze. There is some question in the writer's mind as to the value of this character as an identification mark. The question is now under investigation.

[10] Nichols, Andrew, *Proc. of the Boston Soc. of Nat. History,* Vol. 1, Aug. 2, 1843, p. 136.

[11] Hinckley, Mary C., "On Some Differences in the Mouth Structure of Tadpoles of the Anourous Batrachians Found in Milton, Mass.," *Proc. of the Boston Soc. of Nat. Hist.,* Vol. 21, 1882, pp. 307–314.

Miss Dickerson states that the eggs of *Bufo fowleri* are often arranged in double rows, but that, so far as known, the eggs of *B. americanus* are always laid in single strings. If these characteristics hold true for the two toads it would appear that the toad with which Gage[12] worked was *Bufo fowleri*, rather than *Bufo lentiginosus americanus*. Speaking of the toads with which he worked, Gage states that they lay their eggs from the middle of April until the middle of June, and that the eggs were laid in two strings, one from each oviduct. The lateness of the egg-laying season adds to the probability that Gage worked with *B. fowleri* rather than with *B. americanus*.

From the observations of various observers, it is evident that *Bufo fowleri* is a widely distributed toad and is extremely abundant in many places from New Hampshire, throughout New Jersey, the District of Columbia, southward at least as far as Gwinnett, Jackson and Hall Counties in northern Georgia. Cope (previously cited) records a specimen of this toad from New Harmony, Posey County, Indiana. He also states that a specimen of the variety *B. lentiginosus* var. *americanus* from Nebraska approximates so nearly *B. fowleri*, that the latter can not be regarded as under all circumstances separate and specific in its rank.

Miller and Chapin have found that toads taken on the Palisades and on the northern end of Manhattan Island sometimes show forms intermediate between *B. americanus* and *B. fowleri*. These observers have suggested that such intermediate forms may represent hybrids, but, as they state, it is a question for experimental study.

For a long time the writer has had in mind the question of experimental hybridization between typical forms of *B. fowleri* and *B. americanus*. It would be of considerable interest to determine whether or not these two toads can be hybridized. Although *B. fowleri* is more sensitive to lower temperatures than *B. americanus*, and lays its eggs later in the season, it should not be especially difficult to provide conditions that would bring the mating season of the two toads together under temperature conditions required by *B. fowleri*. It is very probable that the hibernation period of *B. americanus* could be prolonged by artificial refrigeration until the mating and egg-laying period of *B.*

[12] Gage, S. H., "Hibernation, Transformation and Growth of the Common Toad (*Bufo lentiginosus americanus*)," Ithaca, N. Y., *Proc. of Amer. Assoc. for the Advancement of Science*, 47: 1898.

fowleri had arrived. If experimental hybrids could be obtained, it would be especially interesting to compare the voices of the hybrids with the voices of the parents, as well as to determine the hereditary behavior of various other characters.

In those localities where both toads are found, differences in behavior peculiar to each species tend to prevent natural cross mating. *Bufo americanus* is the first toad to appear and, at least around Oxford, Massachusetts, has completed egg-laying and left the water long before *B. fowleri* has appeared. Furthermore, the preference that *B. fowleri* shows for certain ponds from year to year is rather remarkable.

Fowler (letter previously cited) noted that only certain ponds around Danvers, Massachusetts, were visited by *B. fowleri*. In the region of the writer's early home, Oxford, Massachusetts, the same rigid preference was shown for certain bodies of water during the mating season. Here it was indicated that these toads traveled very long distances to reach a certain quiet bend in the Maanixit River. Although other permanent bodies of water were near, these, for some reason, were never visited by these toads.

The writer hopes that an interest in our common toads will finally lead some one to investigate the possibility of experimental hybridization between *B. americanus* and *B. fowleri,* and the question of the relationship of these toads. Batrachian hybridization seems never to have been undertaken. It would appear that such investigations would throw much light on the question of geographic variation, intergrading forms, etc. Few creatures are more companionable and harmless in their behavior and more useful to the agriculturist as insect destroyers, than the toads. Knowledge of their habits, relationship, etc., is not only of scientific, but also of soundly practical interest.

ADDITIONAL REFERENCES IN THE LITERATURE TO FOWLER'S TOAD

Holbrook, J. E. *North American Herpetology*, Vol. 5, 1842. Speaking of *Bufo lentiginosus* Shaw, he says the males seek the females in the month of May when hundreds may be seen together in some stagnant pool depositing their eggs. Of the notes he says: ''The males at this season are extremely noisy, though at other times they are silent, or make only a slight chirp when taken '' (p. 9).

Gorman, Samuel. The North American Reptiles and Batrachians. *Bull. Essex Inst.,* Vol. 16, 1884. On page 42 he says of *B. fowleri* Putnam: ''This is an *americanus* of moderate size and with frontal ridges low,

close together, and nearly or quite parallel. Voice peculiar. Manitoba to Winnipeg; Massachusetts.''

Cope, E. D.   Check List of North American Batrachia and Reptilia with a Systematic List of the Higher Groups and an Essay on Geographical Distribution based on the Specimens contained in the U. S. Nat. Museum. 1875. P. 29, *B. lentiginosus*, subspecies *fowleri* is given a distribution from Massachusetts to Lake Winnipeg. Lists *B. l. fowleri* (page 86) as confined mostly to the Canadian District of the Eastern Region.

Hay, O. P.   The Batrachians and Reptiles of Indiana, 17th Ann. Rept. of Dept. of Geol. and Nat. Resources of Indiana, 1891. *B. fowleri* considered a variety of *B. lentiginosus*. Range given as Danvers, Mass., and the fact that Cope reported a specimen from New Harmony, Indiana (p. 459).

Sherwood, W. L.   The Frogs and Toads Found in the Vicinity of New York City. Abst. No. 10 of the Proc. of the Linn. Soc. of N. Y. for year ending 1898. Mentions *B. fowleri* as a subspecies of the common toad, stating that it was confined to northeastern Massachusetts.

Jordan, David Starr.   A Manual of the Vertebrate Animals of the Northern United States. 1899. On page 182, *B. fowleri* is mentioned as a variety of *B. lentiginosus*.

Ditmars, Raymond L.   The Batrachians of the Vicinity of New York City. *The American Museum Journal*, Vol. 5, 1905. Speaking of the common toad, he says there are four varieties, one of which occurs only in northeastern Massachusetts.

Fowler, H. W.   A Supplementary Account of New Jersey Amphibians and Reptiles. Rept. of New Jersey State Museum, Part III, 1911. *Bufo fowleri* is mentioned.

Hancock, J. L.   The Toad's Social Life in Nature. Sketches in Temperate America, 1911. Fowler's toad is briefly mentioned and illustrations are shown.

Surface, H. A.   First Report on the Economic Features of the Amphibians of Pennsylvania. Zoological Bulletin of the Div. of Zoology, Pennsylvania Dept. of Agriculture, Vol. III, Nos. 3 and 4, May–July, 1913. On page 114, *B. fowleri* is discussed. Statement made that it has been recorded from New England and New York.

H. A. Allard

Washington, D. C.,
May, 1916

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